The Behavioral Role and the Structure of the Aesthetes of Chitons

BY

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(Plates 8 and 9)

INTRODUCTION

Young Amphineura of the order Polyplacophora, commonly called chitons, have long been known to be negatively phototactic. During hours of daylight they are found under and between closely spaced rocks and in other places of relative darkness. Older chitons with worn and encrusted valves seem to be indifferent to the general illumination. The valves of chitons contain numerous sense organs. Due to their gross structure, these organs, called aesthetes, have been assumed to be photoreceptors by most investigators.

I have attempted to determine the function of the aesthetes by electron microscopy, electrical recording of nervous impulses, and behavioral observations.

MATERIALS AND METHODS

BEHAVIORAL

Observations regarding photoreception were made using a beam of light from a dissecting microscope lamp narrowed to an incident beam of approximately 3 mm diameter. The beam of light was directed onto different areas of the animals and their movements were observed.

Mechanoreception of the aesthetes was investigated by observing the movements of the animals while exposing their shell surfaces to touch of dissecting needles and a soft camel's hair brush.

In order to test chemical sensitivity of the chiton's dorsal surface, two thin glass cylinders were wax-sealed to the valves on each side of *Mopalia lignosa* (Gould, 1846). The animals were placed on a marked glass plate and submerged in sea water so that the open end of the glass cylinders remained above the level of the water (Plate 8, Figure 1). Homogenates of the sea star, *Pisaster ochraceus* (Brandt, 1835), and mid-tidal red and green algae were made. The homogenates were placed in contact with the surface of the shell by partially filling the glass cylinder on one side of the animal. The cylinder on the other side was always filled with unaltered sea water. Movement of the animal, toward or away from the side exposed to the homogenates, was observed. In addition to the homogenates, dilute hydrochloric acid was placed in one of the cylinders and the animals were observed.

ELECTRICAL RECORDING

I first attempted to record the mass electrical response from the whole animal with amplifier and oscilloscope. The chiton was immobilized between two chambers filled with sea water with light-shielded electrodes placed in each. Alternate exposure to light and darkness of the dorsal surface of the valves produced no recognizable change in the large slow muscle potentials continually seen. Isolating the anterior valve alone between the two sea-water bathed electrodes likewise produced no pattern related to exposure to light. Glass capillary microelectrodes with tips of approximately 20 μ diameter were placed over individual micraesthete caps and unit recording was attempted. Here again no impulses could be detected. Fine insulated steel recording electrodes were then introduced into the main aesthete nerve canals of broken valves and only photoelectric effects, probably of the elctrode itself, were observed. Due to these repeated failures at electrical recording from the aesthetes I did not pursue this approach further.

ELECTRON MICROSCOPY

Mopalia lignosa (Gould, 1846), *M. hindsii* (Reeve, 1847), and *Tonicella lineata* (Wood, 1815) were the three chiton species used throughout this work. All were ob-

tained along the California coast from Monterey to Point Arena. Shells were broken into fragments of less than 0.5 mm^3 and immediately fixed in 1% osmium tetroxide in sea water for 30 minutes to one hour at 0 to 5° C. Following fixation they were rinsed with sea water and decalcified in a 5% solution of ethylene-dinitrilo-tetra-acetic acid in sea water for periods of 12 to 24 hours. Dehydration was in a graded series of ethanol. Some of the tissues were infiltrated with Araldite and some with Epon. Sections were cut with both diamond and glass knives on a Porter-Blum microtome. Both unsupporting and Formvar-coated copper grids were used. The sections were examined with an Akashi TRS-50El electron microscope.

Potassium permanganate in sea water and acetate-veronal buffered osmium tetroxide fixatives were used, but without success. In addition, double fixation with 1% osmium tetroxide in sea water, before and after decalcification, was done. Here the processing resulted in destruction of much fine structure. Undecalcified shell fragments, fixed, dehydrated and embedded in hard Epon were sectioned with a diamond knife, but the ultrathin sections disintegrated upon the microtome trough. Thicker sections proved to be useless.

RESULTS AND DISCUSSION

Probably all chitons with exposed valves possess organs, called aesthetes, embedded in the shell. These organs are apparently sensory and always occur in two sizes, megalaesthetes and micraesthetes. There are no aesthetes of intermediate size. In addition to the aesthetes, some chitons possess larger organs branching from the same intra-shell nerves, called eyes. These eyes possess a cornea, lens and retina (Moseley, 1884 and 1885). None of the species of chiton on the California coast have been reported to possess eyes, but all species that I have observed or that other investigators have reported on have the two types of aesthetes.

HISTORY

Marshall (1869) found in the shells of chitons canals of two sizes terminating in cup-shaped caps. He regarded the tissue found in these canals as respiratory in function. Van Bemmelen (Hubrecht, 1882) proposed that the organs found in these canals were homologous with the bundle of fibers supporting the spines of the girdle. Moseley (1885) reported that a Dr. W. B. Carpenter first observed the perforate structure of the tegmentum of chitons (date not specified). Moseley examined many alcohol-preserved chitons and found three types of organs: those which he called eyes, and two other similar, but much smaller structures. He named these smaller structures aesthetes. He thought the aesthetes to be organs of touch and the eyes to be photoreceptive organs. Moseley followed the pathways of the tubes leading from the eyes and aesthetes and he was of the opinion that they terminated in the parietal (pallial, branchial) nerves. The size of the eyes found ranged from 188μ to 42μ in diameter. The arrangement of the eyes varied from an irregular scatter to rows, either concentric or radiating to the apex of the tegmentum.

Blumrich (1891) made a microscopic study of the aesthetes of various chitons. He identified these organs as nervous structures and suggested they were probably photoreceptors. Plate (1902) did an extensive study on the general anatomy of the chitons, including the aesthetes, but with apparently no new discoveries regarding the nature of these organs. Nowikoff (1907, 1909) did detailed anatomical work on the aesthetes and was convinced that they were nerves. LeLoup (1940) looked at the chitons of the California coast and noted the occurrence and gross appearance of the aesthetes, but did no microscopic work.

BEHAVIORAL OBSERVATIONS

Photoreception: Crozier & Arey (1918) found that a shadow of a fly 6 feet away caused *Chiton tuberculatus*

Explanation of Plate 8

Mopalia lignosa (Gould, 1846)

Figure 1: Chitons with glass cylinders attached for observations on chemoreception. cyl. - glass cylinders for receiving substances to be presented to the surface of the shell.

Figure 2: Photomicrograph of an aesthete complex. me - megalaesthete; mi - micraesthete; cv - clear vacuoles; s - surface of shell. (x 590)

Figure 3: Electron micrograph of a longitudinal section through the megalaesthete cap. tu - tubes of cap; mc - megalaesthete cone material. (x 5500)

Figure 4: Electron micrograph of a cross section of the megalaesthete nerve between or below the point of micraesthete branching. cv - clear vacuoles; nu - nucleus. (x 7500)

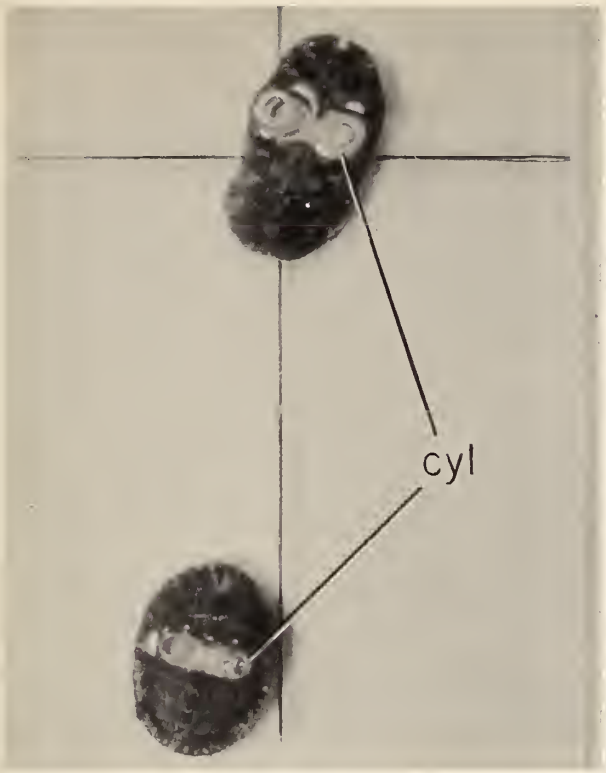

Figure 1

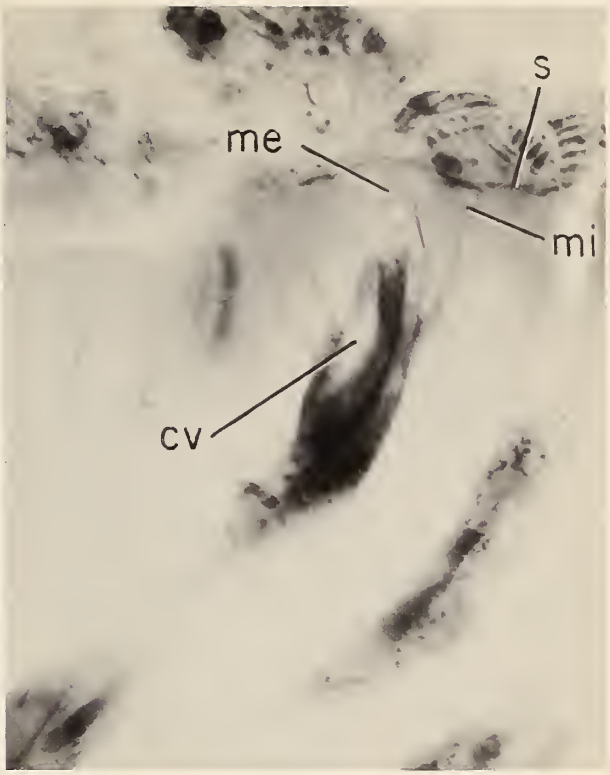

Figure 2

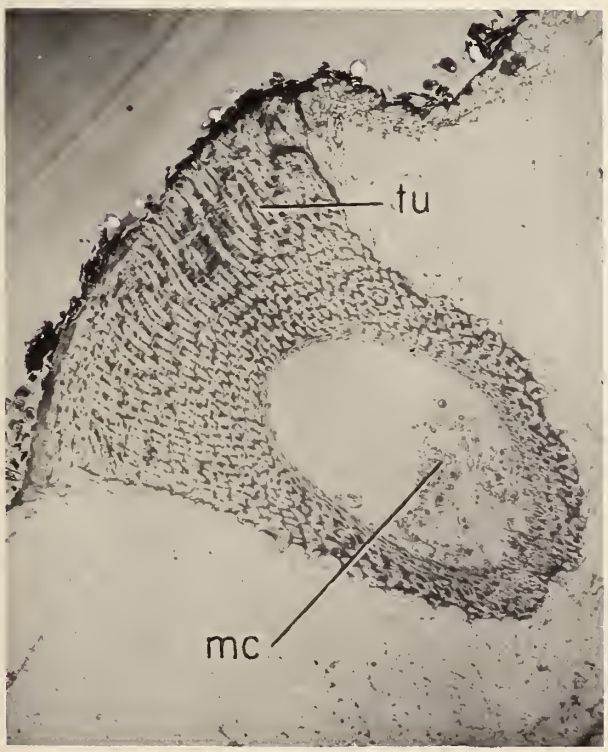

Figure 3

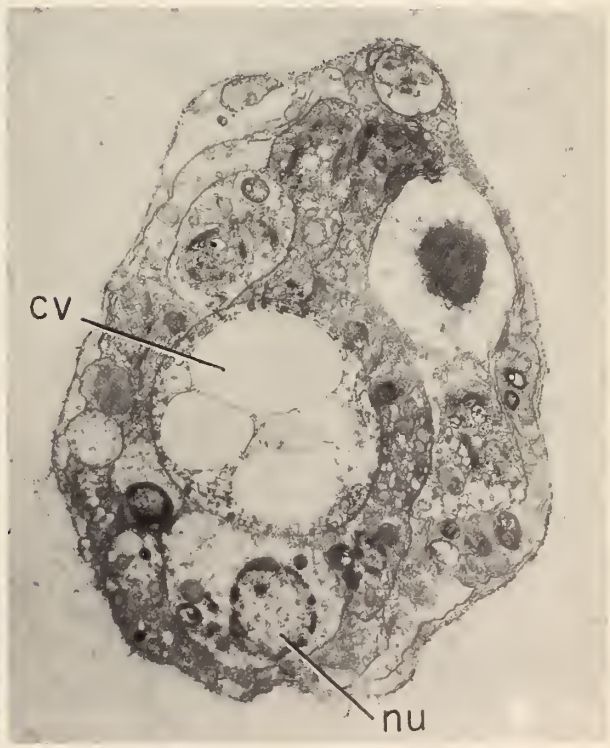

Figure 4

photographs by PAUL OMELICH

Linnaeus, 1758, to halt motion temporarily! These authors stated that a miscellaneous collection of individuals may be caused to separate into two general size groups by sunlight, the larger moving into, the smaller away from lighted areas. The periphery of the girdle was found to be sensitive to a light increase, but the reaction was not oriented. Only a depression of the girdle to the substrate occurred. When they amputated the girdle the smaller chitons still oriented away from the light while the older ones went toward it.

Heath (1899) observed the chitons of Monterey Bay, California and found that they remain out on their feeding ground only when the day is foggy or dark. He observed that *Ischnochiton magdalenensis* (= *I. heathiana* Berry, 1946) adults are found under rocks during the day and only come out to feed at night.

Crozier (1921) found an almost perfect correlation between the degree of erosion of chitons and the relative illumination of the situations frequented. Uneroded chitons were found to be photonegative even to moonlight. They were photopositive or indifferent when completely eroded. He found that the age of the animal was not important, only the degree of erosion.

Douglas (1952) observed that the normal negative response to light exhibited by *Cyanoplax dentiens* (Gould, 1846) depends upon the presence of all or most of its aesthetes. He removed the aesthetes of young photonegative *C. dentiens* by eroding their valves with emery cloth. These chitons were then often found in the direct rays of the sun along with older, naturally eroded animals.

I exposed portions of *Mopalia lignosa* to illumination from a narrow beam of light from a dissecting microscope lamp. A direct beam to any part of the girdle produced a clamping of the girdle to the substrate similar to that observed by other investigators. Illumination to one side of the valves only caused the animals to move away from the light. Illumination of either the anterior or posterior ends likewise produced a negative taxis.

It is remotely possible that the actual photoreceptors of chitons are not contained within the shell, but are located in the tissue beneath, the light being transmitted through the dense calcium carbonate of the valves. But this seems very improbable due to the relatively low intensities of light necessary to produce an oriented response. When only diffuse sunlight is allowed to enter through a slot into a blackened wooden box containing chitons, orientation is obtained (Arey & Crozier, 1919) and the animals orient even to moonlight (Crozier, 1921). It seems likely, therefore, that photoreception does occur in the shell of chiton. Because the aesthetes are the only nervous structures found within the shells it is quite probable that they are photoreceptive.

Mechanoreception: Moseley (1885) believed that the aesthetes were tactile organs. He thought this on the basis of the slightly protruding caps which he observed on the aesthetes. Arey & Crozier (*op. cit.*) state regarding the aesthetes, "Definite evidence as to their functional significance has been completely lacking." They did comprehensive tactile, chemical and light behavioral work on *Chiton tuberculatus*. They concluded that in this species "There are no tactile receptors in the shell plates." But regarding another kind of chiton they observed minute projecting hairs on the valves, and they stated (without describing the methods used), "We find that the tegmentum of *Ischnochiton purpurascens* (Adams, 1845) is very sensitive to touch."

I tested *Mopalia lignosa, M. hindsii* and *Tonicella lineata* for mechanoreception. Lightly dragging a steel dissecting needle across the surface of the shells consistently produced a clamping reaction of the girdle to the substrate. But brushing with a camel's hair brush produced no effect. It seems likely that the response found as a result of touching with the hard steel needle is probably a result of shell distortion or vibration, or both, being transmitted to the foot and girdle where no doubt mechanoreceptors are found.

My longitudinal sections of the megalaesthete caps show them often slightly elevated above the surface of the shell (Plate 8, Figure 3). The micraesthete caps are consistently depressed. Moseley (1885) and Blumrich (1891) showed both the megal- and micraesthetes slightly elevated from the surface. Nowikoff (1909) saw them level with the shell. The position of the caps higher than that of the shell surface is not a prerequisite to mechanoreception, but touch receptors firmly fixed on their peripheries to the hard calcium carbonate of the shell and not protruding above it would most likely operate by recording minute distortions of the shell as do campaniform sensilla of the insect cuticle. But this does not seem likely due to the extreme hardness of the valves.

While this investigation did not conclusively prove that either the megalaesthetes or the micraesthetes are organs of touch, it seems to me that it is very unlikely that they function in this way.

Chemoreception: Barnawell (1960) found that in their natural habitat several species of chitons of the same types which I used eat food in the following order of total quantity:

1. Algae (red and brown)
2. Diatoms
3. Bryozoa
4. Hydroids
5. Barnacles
6. Sponges and mollusks

Feder (1959) studied the food of the starfish *Pisaster ochraceus* (Brandt, 1835) and stated that *Pisaster* was observed to feed primarily on gastropods and chitons.

Thinking that exposure of the surface of the valves of chitons to extracts of their most favored food and their common predator might reveal the possession of chemoreceptive function of the aesthetes, I applied homogenates of algae and starfish to the surface of one side of the shell only, one substance at a time (Plate 8, Figure 1). The glass cylinders containing the materials protruded above the surface of the water so that none of the homogenates could reach any other receptor areas of the animals. The cylinder opposite the food- or predator-filled one was filled to the same level with sea water from the chiton's habitat tank. Exposure of the shells to algae, starfish extract and dilute hydrochloric acid did not produce a visible response. Then I dropped several drops of the algae onto the girdle on one side only. Again there was no response. But when I dropped the starfish extract onto the mantle the animals initiated motion anteriorly and slightly away from the exposed side. Dilute hydrochloric acid evoked the same responses as the starfish extract, only more pronounced. These experiments were repeated a sufficient number of times to justify the conclusion that it is improbable that the aesthetes are chemoreceptive organs for the materials tested.

Microscopy: Invertebrate photoreceptors are much more diverse than vertebrate ones. They comprise several different structural types with only a few common features. Electron microscope studies have generally concentrated on the "higher" invertebrate phyla. Much work on the arthropod ommatidia and the cephalopod retina has been done. Aside from the Cephalopoda little work has been done on mollusks. I think that this investigation is the first attempt to ascertain the fine structure of the presumed photoreceptors in the Amphineura. The aesthetes of chitons are thought to be photoreceptors only because of behavior and gross structure. Therefore I prepared these organs for electron microscopy.

The invertebrate photoreceptor region is generally composed of cells containing organelles called rhabdomeres. These rhabdomeres in mollusks and in many other invertebrates contain pigment granules. In addition, the rhabdomeres are composed of an array of tubules and microvilli probably derived from cell membranes. The average rhabdomere tubule is approximately 600 Å in diameter (Moody, 1964). Eakin (1963) adds the generalizations that many mitochondria are situated near the light sensory apparatus and that an axon-like fiber leads from the basal end of the receptor cell. Eakin (*op. cit.*) states that in the annelid-arthropod-mollusk complex a fibrillar and centriolar assembly like that of the vertebrate photoreceptor cilium has not been seen. Miller (1958) found an exception to this generalization in the ocellus of the scallop *Pecten irradians* Lamarck, 1819. Here he found ciliary filaments leading into the bases of the lamellae.

Miller (1960) described the tubular units of rhabdomeres in general as varying in diameter from 0.04 - 0.12μ. He looked at *Pecten* ocelli (1958) and described globular appendages that are derived from cilia. Irregular matted microvilli extended from the photoreceptor cell. The diameter of these microvilli tubules was approximately 0.07μ.

Eakin (1963) studied the eye of the garden snail *Helix aspersa* Müller, 1776, and found the photoreceptor cell studded with microvilli, each about 0.1μ in diameter. The microvilli are radially arranged parallel to the long axis of the cell. They extend as much as 12μ to the surface of a large structureless lens. No ciliary or centriolar apparatus was found.

Wolken (1958) looked at the retinal structure of *Octopus* and *Sepia*. He found the retina made up of rhabdomes analogous to those of the arthropod eyes. Each rhabdome consists of four visual units radially arranged. These units contained regularly arranged tubules approximately 0.05μ in diameter. A central space containing pigment cells with screening pigment granules separated the rhabdomes. Zonana (1961) saw *Sepia* microvilli of an average diameter of 0.1μ.

In this investigation light microscopy showed that the general arrangement of the aesthetes of *Mopalia lignosa* is similar to that found in chitons by other researchers in that two types of organs are present, the smaller micraesthetes branching from the megalaesthete (Plate 8, Figure 2).

Electron microscopy revealed some new detail. The megalaesthete cap in longitudinal section (Plate 8, Figure 3) shows a regular tubular honeycomb extending from the surface of the shell almost to the center cavity. This

Explanation of Plate 9

Mopalia lignosa (Gould, 1846)

Figure 5: Electron micrograph of a longitudinal section of the aesthete nerve below the branching of the micraesthetes. nf - neurofibrils; pi - pigment granules. (x 20000)

Figure 6: Electron micrograph of a longitudinal section of the aesthete nerve farther down than that of Figure 5. nf - neurofibrils; pi - pigment granules. (x 22000)